FINAL REPORT

South Carolina State Wildlife Grant SC-T-F13AF01183

South Carolina Department of Natural Resources

October 1, 2013 – September 30, 2016

Project Title: Evaluation and Monitoring of the Santee River Basin Robust Redhorse Restoration Effort

Project Goal and Objectives:

The overall goal of our proposed project was to initiate the genetic evaluation and monitoring phase of the Santee River robust redhorse restoration effort. Through the genetic evaluation of both adults and juveniles, we were to assess the contribution of each stocked year class, temporal recruitment patterns, age distribution, and genetic health of the Santee River Basin robust redhorse population. The project objectives were:

1. Conduct annual surveys for robust redhorse within the Santee River Basin.
2. Determine the contribution of each stocked year class.
3. Evaluate temporal recruitment patterns and age distribution of the population.
4. Evaluate the genetic health of the new robust redhorse population.

Objective: 1. Conduct annual surveys for robust redhorse within the Santee River Basin.

Accomplishments:

Annual collection electrofishing surveys were conducted in areas of the Upper Broad River, Lower Broad River, and the Wateree-Congaree rivers were sampled by surveys. Prior to the onset of the spring surveys, a gravel bed survey of the Broad River section below Parr Dam was completed during the winter 2013-2014 months when water levels were lower (~1,000 cfs). Appropriate spawning habitat in terms of both gravel bar characteristics and water depth during typical springs (3-4,000 cfs) were identified. Spring 2014 water levels were uncharacteristically high and resulted in little access to robust redhorse during typical collection on spawning gravel beds, so collection numbers were lower in 2014 (Table 1).

Electrofishing surveys were completed both below and above Parr Dam in the Broad River during the spring as well as the fall in association with a small mouth bass depletion survey (6 boats) in both areas. A total of 13 fish were sampled in the Broad during 2014. Although monitoring of fish passage at the Columbia Dam was not possible due to the high spring waters, four robust redhorse were detected moving through later in the season. Collections in the Wateree River resulted in a total of 25 robust redhorse during 2014. Individuals from both the Broad and Wateree river systems represented multiple year classes of recruitment based on size and coded-wire tag (CWT) location. During August 2014, a juvenile robust redhorse was collected in the Santee River – representing the first collection of a juvenile within the Santee system.

Table 1. 2014 field collections of robust redhorse in the Santee system.

Sub-basin	Area	Survey	Number of Robust Redhorse
Broad River	Above Parr	Spring	2 adults
		Fall	6 adults
	Below Parr	Spring	5 adults
		Fall	0 adults
Wateree River	Tailwaters	Spring	23 adults
Santee River		Summer	1 juvenile

During 2015, areas of the Upper Broad River, Lower Broad River, and the Wateree-Congaree rivers were sampled by electrofishing surveys, resulting in 2, 18, and 2 robust redhorse being collected, respectively.

During 2016, robust redhorse population monitoring included both directed effort and incidental observations. Three days of directed effort were expended in June 2016 on the Upper Congaree, Lower Broad, and Upper Broad River segments. No Robust Redhorse were collected in the Congaree or Upper Broad River during these surveys. However, 6 fish were collected in the Lower Broad River segment. Fin clips were taken from all individuals and 5 individuals received new PIT tags. Coded wire tags were not detected in three fish and the absence of tags in the two smallest fish suggested the possibility that they were naturally recruited.

Observations collected in the study area during other monitoring projects have been helpful in expanding our data collection. In April 2016, an electrofishing survey in the Upper Broad River by SCANA captured two male robust redhorse in spawning condition. One fish was measured, photographed and released before it could be checked for internal tags or a tissue sample taken. However, a PIT tag was detected in the other capture and a tissue sample was obtained. This individual had been captured twice in successive years, during fall sampling about 10 river miles downstream. Three adult Robust Redhorse, in spawning condition, were observed during Duke Energy's spring monitoring below Wateree Dam. Tissue samples were obtained from all three specimens. DNR fall monitoring in the lower Saluda River (Congaree/Wateree river segment) detected two robust redhorse and a tissue sample was collected for one fish. Fish passage monitoring at the Columbia Fishway detected one fish, though conditions were poor following the floods of October 2015.

All fish sampled during the project were checked for the presence of coded-wire tags (CWT) and fin clipped for genetic analysis, unless noted otherwise. Fin clips were stored in 99% ethanol or sarcosyl-urea preservative solution (8M urea, 1% sarcosyl, 20 mM sodium phosphate, 1mM EDTA) and transferred to the SCDNR Population Genetics lab for processing and archiving.

Significant deviations: There were no significant deviations for this objective.

Objective: 2. Determine the contribution of each stocked year class (YC).

Accomplishments:
Genetic processing of all field collected robust redhorse (n=74) and the 2009-2011YC hatchery samples (n=45) have been completed during this grant. Wizard SV Genomic DNA Purification System kits (Promega Corp.; Madison, WI, USA) were used to isolate the DNA from ethanol preserved fin clip tissues and a Sera-Mag Speed Beads (Thermo Fisher Scientific, Waltham, MA) isolation protocol was used to isolate DNA from sarcosyl-urea preserved samples. Following DNA isolation, all samples were amplified in three multiplexed polymerase chain reactions (PCR) using 10 microsatellite loci as described in Darden and Tarpey (2014). All amplifications were performed in 11 µL reaction volumes in BIORAD iCyclers and run with two negative controls. PCR products were separated and visualized on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter; Brea, CA); 1 µL of PCR product was denatured using 40 µL of a 400 bp size standard mixed with sample loading solution. Resulting chromatograms were scored using CEQ Fragment Analysis Software (Beckman Coulter; Brea, CA) to determine allele size. Chromatograms were read and scored by two independent readers for quality assurance. If agreement between scores was not reached, scores were not included in further analyses. Persistent problems amplifying Mro306 led to this locus being dropped from all further processing and analyses, leaving nine multiplexed loci.

Of the hatchery fish, all 45 were successfully genotyped and are archived to test for recaptures.

To determine whether field collected fish were of hatchery or wild origin, genotypes were compared to the 2004–2013 robust redhorse broodstock using CERVUS 3.0 parentage analysis software (Kalinowski et al. 2007) and also tested for recaptures against the 45 known hatchery releases using CERVUS 3.0 identity analysis.

One collected fish, suspected to be a shorthead redhorse at capture, did not amplify with our marker set indicating that it was indeed a shorthead redhorse, and this sample is removed from all further calculations. Overall, 43 fish were successfully assigned to seven YC based on genetic results and/or CWT placement (Table 2). There were six recaptures within the dataset. Twenty-four fish could not be assigned to year classes based on genetics or CWT due to genetic broodstock data gaps. Based on size, 20 of these 24 samples are most likely progeny of the 2005–2007 broodstock, for which not all genetic samples are available. The remaining four fish are of the 2011–2013 YC based on size. Given that we can completely track those YCs genetically and these fish did not match any broodstock parents, these represent the first four wild-spawned progeny in our collections from the Santee River system, two of which were captured in the Wateree-Congaree segment and two of which were captured in the Broad River. The presence of successful wild spawning and recruitment in the Santee River is a critical achievement in establishing a self-sustaining population.

Significant deviations:
There were no significant deviations for this objective.

Table 2. Year class assignment based on genetic results and coded wire tag detections for robust redhorse collected in the Santee River Basin.

Designation	Number Captured
Cultured	43
2004 YC	10
2005 YC	9
2007 YC	1
2008 YC	4
2009 YC	16
2010 YC	1
2013 YC	2
Unassigned	20
Wild	4
Recapture	6
Total	**73**

Objective: 3. Evaluate temporal recruitment patterns and age distribution of the population.

Accomplishments:
Eight of the 9 stocked YC were represented in the analyzed samples (Table 3), with the number of robust redhorse assigned to each YC significantly correlated with the number stocked ($p = 0.007$; Pearson's $r = 0.893$). Equal recruitment among YC is beneficial as there is no over-representation by individual parental crossings or complete failure of YCs. The most recent year classes (2010–2013) have not been at-large for long and are likely not fully recruited to the sampling protocols yet, which are mostly dependent on sampling adults while on the spawning grounds. In the future, we will assess the growth trajectory of the new population to determine if it appears to be increasing, stable, or decreasing.

Significant deviations:
There were no significant deviations for this objective.

Table 3. Overall year class summary using genetics, coded wire tags, and total lengths to assign to YCs.

Designation	Number Captured	Stockings	
		Phase	Number
Cultured	63		
2004 YC	10	I, II, III	21,330
2005-2007 YC	30	I	34,235
2008 YC	4	I	1,383
2009 YC	16	I, III	9,001
2010 YC	1	III	20
2011 YC	0	III	23
2013 YC	2	I	5,941
Recapture	6		
Wild-Spawned	4		
Total	**73**		

Objective: 4. Evaluate the genetic health of the new robust redhorse population.

Accomplishments:
We calculated basic molecular diversity indices for each locus using ARLEQUIN ver. 3.5 (Excoffier and Lischer 2010), including allelic size range (R), allelic richness (A), observed heterozygosity (H_O), and gene diversity (H_E; Nei 1987). Inbreeding coefficients (F_{IS}; Weir and Cockerham 1984) were calculated using GENEPOP (Rousset 2008). A contemporary effective population size (Ne) was then estimated using the linkage disequilibrium method as implemented in LDNe with a 0.02 allele frequency exclusion criterion, and 95% CI for parametric estimation of Ne (Waples and Do 2008).

Genetic population metrics were compared between the field collected samples in the Santee River and the Savannah River and Pee Dee River (Darden and Tarpey 2014). Allelic richness, allelic range, expected heterozygosity, and observed heterozygosity were all similar between the Santee and Savannah (Table 4), which is a positive sign for the Santee population since the Savannah is considered the healthiest known population of robust redhorse and was the source population for the Santee. All three populations have inbreeding coefficients near zero, which indicates no inbreeding is occurring. The effective population size in the Santee was estimated with the current sample set to be 50.1, compared to 156.3 in the Savannah. However, a reduced Ne is not unexpected since the largest theoretical effective population size of those in the Santee is limited by the number of broodstock pairings (maximum theoretical hatchery Ne = 99.87) and it should increase as the remaining stocked year classes recruit to the sampling gear and sample size in the Santee increases. Overall, all of the genetic metrics are indicative of a healthy population being restored to the Santee River Basin that closely resembles the source population in the Savannah River. The documentation of wild-spawned juveniles in the Santee River is a significant milestone in the restoration program.

Significant deviations:
There were no significant deviations for this objective.

Table 4**.** Genetic population metrics across nine loci for 57 field caught samples in the Santee River. Statistics for the Savannah River, SC and Pee Dee River, NC are provided by comparison (Darden & Tarpey 2014). Numbers in parentheses represent 95% confidence intervals for Ne, and standard deviations for the other metrics.

Metric	Santee River	Savannah River	Pee Dee River
Sample Size	57	189	55
$Ne_{[LD]}$	50.1 (41.0-62.8)	156.3 (133.1-186.7)	9.7 (8.3-11.1)
Allelic Richness (A)	13.0 (±2.6)	13.0 (±2.8)	9.38 (±2.2)
Allelic Range (R)	16.1(±3.3)	15.7 (±1.6)	12.6 (±5.2)
Expected Heterozygosity (H_E)	0.858 (±0.058)	0.870 (±0.047)	0.806 (0.058)
Observed Heterozygosity (H_O)	0.835(±0.080)	0.891 (±0.046)	0.789 (±0.083)
Inbreeding Coefficient (F_{IS})	0.027	-0.025	0.021

Estimated Federal Cost: $83,155 for the 3 year project; all project funds have been expended.

Recommendations: The grant has been completed; our recommendation is to close the grant.

Prepared by: Daniel Farrae and Tanya Darden; December 13, 2016
Literature Cited:

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